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PATENT & TRADEMARK OFFICE

English Translation of JP-A-2000-1541

[Title of the Invention]

AMINO GROUP-CONTAINING POLYOXYALKYLENE COMPOUND

[Abstract]

[Problem]

To provide an amino group-containing polyoxyalkylene compound which is used for modification of a phospholipid for the purpose of not only reduction of the antigenicity of a compound or a drug and stabilization thereof but also extension of the residence time thereof in a body and wherein a fat emulsion or a liposome using the modified phospholipid has a low toxicity and by-products are formed in small amounts.

[Means for Solution]

An amino group-containing polyoxyalkylene compound represented by the formula (1):
wherein R^1 is a hydrogen atom, a hydrocarbon group having 1 to 24 carbon atoms or an acyl group having 1 to 24 carbon atoms; R^2 is a hydrocarbon group having 3 to 4 carbon atoms; R^3 is a hydrocarbon group having 1 to 10 carbon atoms; AO is an oxyalkylene group having 3 to 4 carbon atoms; n means an average added mole number of the oxyethylene group and is from 1 to 1,000; m means an average added mole number of the oxyalkylene group having 3 to 4 carbon atoms; n/(n+m) is 0.8 or more; and an

addition mode of the oxyethylene group and the oxyalkylene group having 3 to 4 carbon atoms may be a block form or a random form.

[Claims]

[Claim 1] An amino group-containing polyoxyalkylene compound represented by the formula (1):

$$\begin{array}{l} CH_2\text{-}O(CH_2CH_2O)_n(AO)_m R^1 \\ | \\ CH\text{—}O(CH_2CH_2O)_n(AO)_m R^1 \\ | \\ CH_2\text{-}O\text{-}R^2\text{—}S\text{—}R^3\text{—}NH_2 \end{array} \qquad (1)$$

wherein R^1 is a hydrogen atom, a hydrocarbon group having 1 to 24 carbon atoms or an acyl group having 1 to 24 carbon atoms; R^2 is a hydrocarbon group having 3 to 4 carbon atoms; R^3 is a hydrocarbon group having 1 to 10 carbon atoms; AO is an oxyalkylene group having 3 to 4 carbon atoms; n means an average added mole number of the oxyethylene group and is from 1 to 1,000; m means an average added mole number of the oxyalkylene group having 3 to 4 carbon atoms; n/(n+m) is 0.8 or more; and an addition mode of the oxyethylene group and the oxyalkylene group having 3 to 4 carbon atoms may be a block form or a random form.

[Detailed Description of the Invention]

[0001]

[Technical Field to which the Invention Belongs]

The present invention relates to a polyoxyalkylene compound having polyoxyalkylene chains at α- and β-positions of glycerin and having an amino group at γ-position thereof. More specifically, it relates to a

polyoxyalkylene compound having a terminal amino group, which is mainly used in pharmaceutical applications, e.g., polyoxyalkylene-modification of polypeptides, physiologically active proteins, enzymes, and the like and modification of polyoxyalkylene groups in drug delivery systems such as fat emulsions, liposomes, and the like.

[0002]

[Prior Art]

Heretofore, compounds wherein a terminal hydroxyl group of a polyoxyalkylene glycol is replaced by an amino group are described as a lubricating oil (JP-B-54-745854) or as an additive for synthetic resins (JP-A-57-36115) and have been widely utilized. Recently, polyoxyalkylene compounds have attracted attention as important carriers for drug delivery systems and studies have also been actively carried out on compounds wherein an amino group or a carboxyl group is introduced into polyoxyalkylene compounds. Of these, as a compound having two polyoxyalkylene chains, there is known 2,4-bis(O-methoxypolyethylene glycol)-6-chloro-S-triazine wherein a triazine ring intervenes (hereinafter referred to as "activated PEG2") as shown in JP-A-3-72469. Moreover, a polyoxyalkylene compound having a large number of amino groups at side chains of a polyoxyalkylene group is also known (JP-A-8-48764).

[0003]

[Problems that the Invention is to Solve]

Particularly, in a fat emulsion or a liposome using a phospholipid modified with a polyoxyalkylene compound, not only reduction of antigenicity (imunoreactivity) and stability of an included drug but also an effect of extending the residence time in a body are obtained. However, with regard to these conventional amino group-containing polyoxyalkylene compounds, for example, in the case of single-chain polyoxyalkylene compounds containing a terminal amino group, there frequently exist the cases that, when objective substances are modified with them, the effect of extending the residence time in a body inherent to polyoxyalkylene is not sufficiently achieved since they are single-chain compounds. Moreover, since the aforementioned activated PEG2 possesses a triazine ring, there is a possibility of appearance of toxicity when it is administered into a body as a pharmaceutical. Furthermore, with regard to the compound having a number of amino groups at side chains of the polyoxyalkylene skeleton, the modification reaction is difficult to control because there exist a large number of reaction sites, and hence it is difficult to obtain a single compound.

[0004]

An object of the invention is to provide an amino group-containing polyoxyalkylene compound which is used for modification of a phospholipid for the purpose of not only reduction of antigenicity of a compound or a drug and stabilization thereof but also extension of the residence time thereof in a body and wherein a fat emulsion or a liposome using the modified phospholipid has a low toxicity and by-products are formed in small amounts.

[0005]

[Means for Solving the problems]

As a result of extensive studies for solving the above problems, the present inventors have found that the above object can be achieved by a polyoxyalkylene compound having polyoxyalkylene chains at α- and β- positions of glycerin and having an amino group at γ-position thereof, and thus they have accomplished the invention. Namely, the invention is an amino group-containing polyoxyalkylene compound represented by the formula (1):

[0006]

$$\begin{array}{l} CH_2\text{-}O(CH_2CH_2O)_n(AO)_m R^1 \\ | \\ CH\text{—}O(CH_2CH_2O)_n(AO)_m R^1 \\ | \\ CH_2\text{-}O\text{-}R^2\text{—}S\text{—}R^3\text{—}NH_2 \end{array} \qquad (1)$$

[0007]

wherein R^1 is a hydrogen atom, a hydrocarbon group having

1 to 24 carbon atoms or an acyl group having 1 to 24 carbon atoms; R^2 is a hydrocarbon group having 3 to 4 carbon atoms; R^3 is a hydrocarbon group having 1 to 10 carbon atoms; AO is an oxyalkylene group having 3 to 4 carbon atoms; n means an average added mole number of the oxyethylene group and is from 1 to 1,000; m means an average added mole number of the oxyalkylene group having 3 to 4 carbon atoms; n/(n+m) is 0.8 or more; and an addition mode of the oxyethylene group and the oxyalkylene group having 3 to 4 carbon atoms may be a block form or a random form.

[0008]

[Mode for Carrying Out the Invention]

In the formula (1), the hydrocarbon group having 1 to 24 carbon atoms represented by R^1 includes linear or branched alkyl groups such as a methyl group, an ethyl group, a propyl group, an isopropyl group, a butyl group, an isobutyl group, a tert-butyl group, a pentyl group, an isopentyl group, a hexyl group, an isohexyl group, a 2-ethylhexyl group, an octyl group, an isononyl group, a decyl group, a dodecyl group, an isotridecyl group, a tetradecyl group, a hexadecyl group, an isocetyl group, an octadecyl group, an isostearyl group, an octyldodecyl group, a docosyl group, and a decyltetradecyl group, as aliphatic hydrocarbon groups; and aryl groups such as a

butylphenyl group, a dibutylphenyl group, an octylphenyl group, a dinonylphenyl group, and an α-methylbenzylphenyl group, aralkyl groups such as a benzyl group, and a cresyl group, as aromatic hydrocarbon groups.

[0009]

Moreover, the acyl group having 1 to 24 carbon atoms includes acyl groups derived from acetic acid, propionic acid, butyric acid, isobutyric acid, caprylic acid, 2-ethylhexanoic acid, isononanoic acid, capric acid, lauric acid, myristic acid, palmitic acid, isopalmitic acid, stearic acid, isostearic acid, arachidic acid, behenic acid, palmitoleic acid, benzoic acid, hydroxybenzoic acid, cinnamic acid, gallic acid, and the like. Of these, as R^1, a hydrogen atom and a linear alkyl group having 1 to 4 carbon atoms are preferred. Incidentally, two R^1 are present in the formula (1), but these may be the same or different from each other.

[0010]

The hydrocarbon group having 3 to 4 carbon atoms represented by R^2 includes groups derived from hydrocarbon groups having a polymerizable unsaturated group, preferably linear or branched alkylene groups such as a trimethylene group and an isobutylene group derived from hydrocarbon groups having a double bond, e.g., an allyl group and a methallyl group.

[0011]

The hydrocarbon group having 1 to 10 carbon atoms represented by R^3 includes linear or branched alkylene groups such as a methylene group, an ethylene group, a propylene group, and a trimethylene group, and divalent aromatic hydrocarbon groups such as a phenylene group and a benzyl group. Of these, a methylene group and an ethylene group are preferred.

[0012]

The alkylene part of the oxyalkylene group having 3 to 4 carbon atoms represented by AO may be linear or branched and examples of such an oxyalkylene group include an oxypropylene group, an oxytrimethylene group, an oxybutylene group, an oxytetramethylene group, and the like.

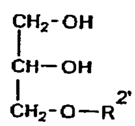
[0013]

n means an average added mole number of the oxyethylene group and is from 1 to 1,000; m means an average added mole number of the oxyalkylene group having 3 to 4 carbon atoms; and n/(n+m) is 0.8 or more, preferably 0.9 or more, more preferably 1.0. When n/(n+m) is less than 1.0, an addition mode of the oxyethylene group and the oxyalkylene group having 3 to 4 carbon atoms may be a block form or a random form.

[0014]

The amino group-containing polyoxyalkylene compound of the invention represented by the formula (1) can be produced, for example, as follows. First, ethylene oxide alone or ethylene oxide and an alkylene oxide having 3 to 4 carbon atoms are added to a compound represented by the formula (2):

[0015]



(2)

[0016]

wherein $R^{2'}$ represents a hydrocarbon group having a polymerizable unsaturated group, preferably a double bond-containing hydrocarbon group having 3 to 4 carbon atoms, such as an allyl group or a methallyl group. At this time, after the addition of ethylene oxide to the compound (2), the alkylene oxide having 3 to 4 carbon atoms may be added, or ethylene oxide and the alkylene oxide having 3 to 4 carbon atoms may be mixed and the addition reaction may be effected at a time. The ratio of the added mole number of ethylene oxide to that of the alkylene oxide having 3 to 4 carbon atoms is determined so that the oxyethylene group should account for 80% or more in order to maintain hydrophilicity of the whole oxyalkylene chain.

[0017]

Specifically, the compound (2) is charged into a reactor, substitution with nitrogen is conducted, and an alkylene oxide (ethylene oxide only or a mixture of ethylene oxide and an alkylene oxide having 3 to 4 carbon atoms) is charged with pressure at 100 to 140°C, followed by reacting them. After the reaction, unreacted alkylene oxide is removed under reduced pressure and the reaction mixture is cooled to 80°C, neutralized by adding an acid such as phosphoric acid or hydrochloric acid, and dehydrated and filtrated to obtain a compound represented by the formula (3'):

[0018]

$$\begin{array}{l} CH_2\text{-}O(CH_2CH_2O)_n(AO)_m H \\ | \\ CH-O(CH_2CH_2O)_n(AO)_m H \\ | \\ CH_2\text{-}O-R^{2'} \end{array} \qquad (3')$$

[0019]

wherein the symbols are as described above. If necessary, by introduction of a hydrocarbon group, e.g., alkylation or acylation of the terminal hydroxyl groups, the compound is converted into a compound represented by the formula (3''):

[0020]

$$\begin{array}{l} CH_2\text{-}O(CH_2CH_2O)_n(AO)_m R^{1'} \\ | \\ CH-O(CH_2CH_2O)_n(AO)_m R^{1'} \\ | \\ CH_2\text{-}O-R^{2'} \end{array} \qquad (3'')$$

[0021]

wherein $R^{1'}$ represents a hydrocarbon group having 1 to 24 carbon atoms or an acyl group having 1 to 24 carbon atoms, and the other symbols are as described above. For example, in the alkylation reaction, an alkylating agent such as an alkyl halide (halogenated alkyl) or an alkenyl halide having a hydrocarbon group represented by R^1 is added in an amount of 1.1 to 3.0 molar equivalents to a hydroxyl group of the compound (3') and the reaction is conducted at 90 to 120°C for 2 to 5 hours, followed by washing with water, removal of unreacted matter, neutralization, dehydration, and filtration.

[0022]

In the acylation reaction, an acylating agent such as an acyl halide or a carboxylic anhydride having an acyl group represented by R^1 is added in an amount of 1.1 to 2.0 molar equivalents to a hydroxyl group of the compound (3') and a dehydration-condensation reaction is conducted at 110 to 140°C for 9 hours in the presence of p-toluenesulfonic acid, followed by treatment with an adsorbent, dehydration, and filtration. In the case that a compound wherein R^1 in the above halide or carboxylic anhydride is an aromatic hydrocarbon group is used, an aromatic hydrocarbon group is introduced. The reaction conditions in this case are in accordance with those in

the above alkylation and acylation. To a thus obtained compound represented by the formula (4):

[0023]

$$\begin{array}{l} CH_2\text{-}O(CH_2CH_2O)_n(AO)_m R^1 \\ | \\ CH\text{—}O(CH_2CH_2O)_n(AO)_m R^1 \\ | \\ CH_2\text{-}O\text{—}R^{2'} \end{array} \qquad (4)$$

[0024]

wherein respective symbols are as described above,
a compound represented by the formula (5):

$HS\text{-}R^3\text{-}NH_2 \cdot HCl$ (5)

wherein R^3 is as described above,
is added in an amount of 1.5 to 10 molar equivalents to the allyl group or methallyl group in the compound (4), and the reaction is conducted in an alcohol such as methanol or ethanol at 30 to 40°C for 3 to 7 hours to introduce an amino group. After completion of the reaction, pH is adjusted to 9 with a 1N NaOH aqueous solution and then the alcohol is removed by evaporation. The reaction mixture is dissolved in a solvent such as chloroform or dichloromethane and then washed with water to remove the unreacted compound (5). Then, after removal of the solvent by evaporation, filtration is conducted to obtain the compound of the formula (1).

[0025]

The amino group-containing polyoxyalkylene compound

of the invention is thought to be chemical modification of a phospholipid, i.e., a basal material for a fat emulsion or a liposome which is one kind of drug delivery systems mainly including an anticancer agent such as adriamycin or cisplatin therein. By the modification with the polyoxyalkylene group, not only stability of the fat emulsion and liposome themselves is enhanced but also an effect of extending the residence time in a blood is expected.

[0026]

[Examples]

The following will describe the present invention in further detail with reference to Examples.

Production Example 1

Into a 5 liter-volume autoclave were charged 66 g (0.5 mol) of glycerin monoallyl ether and 1 g of potassium hydroxide. The atmosphere of the system was substituted with nitrogen gas, followed by temperature elevation to 120°C. Then, 2440 g (55 mol) of ethylene oxide was charged with pressure, followed by 1 hour of reaction at 130±5°C. Then, unreacted ethylene oxide was removed under reduced pressure (200 mmHg, 0.5 hor) while nitrogen gas was allowed to pass through and the whole was cooled to 80°C. Thereafter, pH was adjusted to 7.0 with a 10 wt% hydrochloric acid aqueous solution and dehydration was

conducted at 100±5°C under 100 mmHg for 1 hour. Then, the reaction mixture was cooled to 80°C and the precipitated salt was filtrated off to obtain 2380 g of a compound. The hydroxyl value of the resulting compound was 22.4 (calculated value: 23.0) and the degree of unsaturation was 0.19 (calculated value: 0.2). In this connection, the hydroxyl value was measured in accordance with the method of JIS K-1557 6.4 (1970) and the degree of unsaturation was measured in accordance with the method of JIS K-1557 6.7 (1970). An infrared absorption spectrum of the compound is shown in Fig. 1. Analytical results of gel permeation chromatography (hereinafter referred to GPC) are shown in Fig. 2 and Table 1. Analytical conditions of GPC are as follows.

GPC system: SYSTEM-11 (manufactured by Syowa Denko K.K.)

GPC column: SHODEX KF-804L ×3

Developing liquid: THF

Flow rate: 1 ml/min

Sample concentration: 0.15 wt%

Column oven temperature: 40°C

[0027]

[Table 1]

Table 1

Peak information	Time (minute)	Molecular weight	Height
Start	19.9	20759	2
Top	22.339	5193	34156
End	25.3	1114	0
Number-average molecular weight (Mn)		5131	
Weight-average molecular weight (Mw)		5268	
Polydispersity (Mw/Mn)		1.02673	

[0028]

Results of ^{1}H-NMR spectrum are as follows.

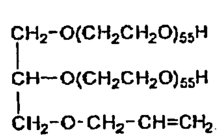
^{1}H-NMR (δ (ppm), CDCl/TMS)

δ=5.2 ppm ($C=C\underline{H_2}$)

δ=5.9 ppm ($-C\underline{H}=$)

From the starting materials, reaction conditions, and analytical values, the resulting compound was deduced to be a compound represented by the formula (6):

[0029]



[0030]

(molecular weight: 5009).

[0031]

Production Example 2

Into a 5 liter-volume autoclave were charged 66 g (0.5 mol) of glycerin monoallyl ether and 0.6 g of potassium hydroxide. The atmosphere of the system was substituted with nitrogen gas, followed by temperature elevation to 100°C. Then, 1340 g (30 mol) of ethylene oxide and 110 g (2 mol) of propylene oxide were weighed into a weighing vessel and were mixed until they formed a homogeneous mixture. The mixture of ethylene oxide and propylene oxide was charged with pressure from the weighing vessel under conditions of 110±5°C and 10 kg/cm^2 or lower over the period of 8 hours. After the charging with pressure, reaction was conducted for 1 hour. Then, unreacted ethylene oxide and propylene oxide were removed under reduced pressure of 200 mmHg for 30 minutes while nitrogen gas was allowed to pass through, followed by cooling to 80°C. Thereafter, pH was adjusted to 7.0 with a 10 wt% hydrochloric acid aqueous solution and dehydration was conducted under conditions of 100±5°C and 100 mmHg for 1 hour. Then, the reaction mixture was cooled to 80°C and the precipitated salt was filtrated off to obtain 1440 g of a compound.

[0032]

The hydroxyl value of the resulting compound was

36.4 (calculated value: 36.2) and the degree of unsaturation was 0.30 (calculated value: 0.32). In this connection, the hydroxyl value and the degree of unsaturation were measured as in Production Example 1. Analytical results of GPC are shown in Fig. 3 and Table 2. Analytical conditions of GPC were as in Production Example 1.

[0033]

[Table 2]

Table 2

Peak information	Time (minute)	Molecular weight	Height
Start	21.2	9539	1
Top	23.341	3082	24069
End	25.6	953	36
Number-average molecular weight (Mn)		2969	
Weight-average molecular weight (Mw)		3061	
Polydispersity (Mw/Mn)		1.03102	

[0034]

From the starting materials, reaction conditions, and analytical values, the resulting compound was deduced to be a compound represented by the formula (7):

[0035]

$$\begin{array}{l} CH_2\text{-}O(CH_2CH_2O)_{30}(CH(CH_3)CH_2O)_2H \\ | \\ CH\text{—}O(CH_2CH_2O)_{30}(CH(CH_3)CH_2O)_2H \\ | \\ CH_2\text{-}O\text{-}CH_2\text{-}CH{=}CH_2 \end{array} \qquad (7)$$

[0036]

(molecular weight: 3082).

[0037]

Production Example 3

Into a 5 liter-volume autoclave were charged 1000 g (0.32 mol) of the compound of the formula (6) obtained in Production Example 2 and 150 g of potassium hydroxide. The atmosphere of the system was substituted with nitrogen gas, followed by temperature elevation to 100°C. Then, 43.5 g (0.84 mol) of methyl chloride was charged thereto under a condition of 100±5°C. After 4 hours of reaction, the whole was cooled to 80°C and unreacted methyl chloride was removed under reduced pressure (200 mmHg or lower) for 0.5 hour while nitrogen gas was allowed to pass through. Then, after 500 g of water was added into the system and the whole was mixed, the mixture was left on standing to be allowed to separate into layers and excess alkali matter of the lower layer was removed. Thereafter, pH was adjusted to 7.0 with a 10 wt% hydrochloric acid aqueous solution and dehydration was conducted under conditions of 100±5°C and 100 mmHg for 1 hour. Then, the reaction mixture was cooled to 80°C and the precipitated salt was filtrated off to obtain 955 g of a compound. The hydroxyl value of the resulting compound was 0.04 (calculated value: 0) and the degree of unsaturation was 0.29

(calculated value: 0.32). In this connection, the hydroxyl value and the degree of unsaturation were measured as in Production Example 1. Analytical results of GPC are shown in Fig. 4 and Table 3. Analytical conditions of GPC were as in Production Example 1.

[0038]

[Table 3]

Table 3

Peak information	Time (minute)	Molecular weight	Height
Start	21.3	9029	2
Top	23.327	3106	25082
End	25.4	1057	28
Number-average molecular weight (Mn)		2987	
Weight-average molecular weight (Mw)		3075	
Polydispersity (Mw/Mn)		1.02931	

[0039]

From the starting materials, reaction conditions, and analytical values, the resulting compound was deduced to be a compound represented by the formula (8):

[0040]

$$\begin{array}{l} CH_2\text{-}O(CH_2CH_2O)_{30}(CH(CH_3)CH_2O)_2CH_3 \\ | \\ CH\text{—}O(CH_2CH_2O)_{30}(CH(CH_3)CH_2O)_2CH_3 \\ | \\ CH_2\text{-}O\text{-}CH_2\text{-}CH{=}CH_2 \end{array} \qquad (8)$$

[0041]

(molecular weight: 3110).

[0042]

Example 1

In a four-neck flask was placed 45.4 g (0.4 mol) of aminoethanethiol ($HSCH_2CH_2NH_2 \cdot HCl$) as a compound of the formula (5), and the temperature was kept at 35±5°C under stirring. Then, 500 g (0.1 mol) of the compound of the formula (6) synthesized in Production Example 1 was dissolved in 500 g of methanol and the resulting solution was added dropwise to the four-necked flask through a dropping funnel over the period of 5 hours. After completion of the dropwise addition of the whole amount thereof, the whole was kept at 40±5°C for further 5 hours to continue the reaction. After the reaction, pH was adjusted to 9 with 1N NaOH aqueous solution. Then, methanol was removed by evaporation at 60±10°C under reduced pressure of 200 mmHg or lower and then the reaction mixture was again dissolved in 1000 g of chloroform. Then, the whole amount thereof was transferred into a separating funnel and was washed with 1 liter of saturated saline three times to remove unreacted aminoethanethiol. Then, chloroform and water were removed by evaporation at 110±10°C under a nitrogen atmosphere under reduced pressure of 50 mmHg or lower and the

precipitated sodium chloride was removed by filtration, whereby 472 g of a compound (molecular weight: 5086) was obtained. The primary amine value of the resulting compound was 11.3 (calculated value: 11.0) and the degree of unsaturation was 0.01 (calculated value: 0).

[0043]

In this connection, the primary amine value is a value obtained by subtracting a secondary amine value and a tertiary amine value determined by titration with hydrochloric acid after reaction between salicylaldehyde and the sample from a total amine value determined by titration with hydrochloric acid. The acid value was measured in accordance with the method of JIS K-1557, 6.6 (1970). The degree of unsaturation was measured as in Production Example 1. An infrared absorption spectrum is shown in Fig. 5. Results of ^{1}H-NMR spectrum are as follows.

^{1}H-NMR (δ (ppm), CDCl/TMS)

δ=1.85 ppm ($-O-CH_2C\underline{H_2}CH_2-S-CH_2CH_2-NH_2$)

δ=2.75 ppm ($-O-CH_2CH_2C\underline{H_2}-S-CH_2CH_2-NH_2$)

δ=2.65 ppm ($-O-CH_2CH_2CH_2-S-C\underline{H_2}CH_2-NH_2$)

δ=3.40 ppm ($-O-CH_2CH_2CH_2-S-CH_2C\underline{H_2}-NH_2$)

From the starting materials, the reaction conditions, and the above analytical values, the resulting compound was deduced to be a compound represented by the formula (9):

[0044]

$$
\begin{array}{l}
CH_2\text{-}O(CH_2CH_2O)_{55}H \\
| \\
CH\text{—}O(CH_2CH_2O)_{55}H \\
| \\
CH_2\text{-}O\text{-}CH_2CH_2CH_2\text{—}S\text{—}CH_2CH_2\text{—}NH_2
\end{array}
\qquad (9)
$$

[0045]

(molecular weight: 5086).

[0046]

Example 2

In a four-neck flask was placed 40.0 g (0.4 mol) of aminomethanethiol ($HSCH_2NH_2 \cdot HCl$) as a compound of the formula (5), and the temperature was kept at 35±5°C under stirring. Then, 500 g (0.1 mol) of the compound of the formula (6) synthesized in Production Example 1 was dissolved in 500 g of methanol and the resulting solution was added dropwise to the four-necked flask through a dropping funnel over the period of 5 hours. After completion of the dropwise addition of the whole amount thereof, the whole was kept at 40±5°C for further 5 hours to continue the reaction. After the reaction, pH was adjusted to 9 with 1N NaOH aqueous solution. Then, methanol was removed by evaporation at 60±10°C under reduced pressure of 200 mmHg or lower and then the reaction mixture was again dissolved in 1000 g of chloroform. Then, the whole amount thereof was transferred into a separating funnel and was washed with 1

liter of saturated saline three times to remove unreacted aminoethanethiol. Then, chloroform and water were removed by evaporation at 110±10°C under a nitrogen atmosphere under reduced pressure of 50 mmHg or lower and the precipitated sodium chloride was removed by filtration, whereby 472 g of a compound (molecular weight: 5074) was obtained. The primary amine value of the resulting compound was 11.4 (calculated value: 11.0) and the degree of unsaturation was 0.01 (calculated value: 0). In this connection, the primary amine value and the degree of unsaturation were measured as in Production Example 1. Results of ^{1}H-NMR spectrum are as follows.

^{1}H-NMR (δ (ppm), CDCl/TMS)

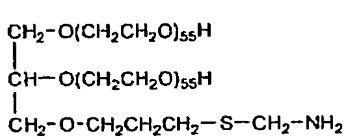
$\delta=1.85$ ppm ($-O-CH_2C\underline{H_2}CH_2-S-CH_2-NH_2$)

$\delta=2.75$ ppm ($-O-CH_2CH_2C\underline{H_2}-S-CH_2-NH_2$)

$\delta=3.74$ ppm ($-O-CH_2CH_2CH_2-S-C\underline{H_2}-NH_2$)

From the starting materials, the reaction conditions, and the above analytical values, the resulting compound was deduced to be a compound represented by the formula (10):

[0047]



(10)

[0048]

(molecular weight: 5074).

[0049]

Example 3

In a four-neck flask was placed 72.6 g (0.64 mol) of aminoethanethiol ($HSCH_2CH_2NH_2 \cdot HCl$) as a compound of the formula (5), and the temperature was kept at 35±5°C under stirring. Then, 500 g (0.16 mol) of the compound of the formula (7) synthesized in Production Example 2 was dissolved in 500 g of methanol and the resulting solution was added dropwise to the four-necked flask through a dropping funnel over the period of 5 hours. After completion of the dropwise addition of the whole amount thereof, the whole was kept at 40±5°C for further 5 hours to continue the reaction. After the reaction, pH was adjusted to 9 with 1N NaOH aqueous solution. Then, methanol was removed by evaporation at 60±10°C under reduced pressure of 200 mmHg or lower and then the reaction mixture was again dissolved in 1000 g of chloroform. Then, the whole amount thereof was transferred into a separating funnel and was washed with 1 liter of saturated saline three times to remove unreacted aminoethanethiol. Then, chloroform and water were removed by evaporation at 110±10°C under a nitrogen atmosphere under reduced pressure of 50 mmHg or lower and the

precipitated sodium chloride was removed by filtration, whereby 470 g of a compound (molecular weight: 3159) was obtained. The primary amine value of the resulting compound was 17.9 (calculated value: 17.8) and the degree of unsaturation was 0.02 (calculated value: 0). In this connection, the primary amine value and the degree of unsaturation were measured as in Production Example 1. Results of ^{1}H-NMR spectrum are as follows.

^{1}H-NMR (δ (ppm), CDCl/TMS)

δ=1.85 ppm ($-O-CH_2C\underline{H_2}CH_2-S-CH_2CH_2-NH_2$)

δ=2.75 ppm ($-O-CH_2CH_2C\underline{H_2}-S-CH_2CH_2-NH_2$)

δ=2.65 ppm ($-O-CH_2CH_2CH_2-S-C\underline{H_2}CH_2-NH_2$)

δ=3.40 ppm ($-O-CH_2CH_2CH_2-S-CH_2C\underline{H_2}-NH_2$)

From the starting materials, the reaction conditions, and the above analytical values, the resulting compound was deduced to be a compound represented by the formula (11):

[0050]

$$
\begin{array}{l}
CH_2-O(CH_2CH_2O)_{30}(CH(CH_3)CH_2O)_2H \\
| \\
CH-O(CH_2CH_2O)_{30}(CH(CH_3)CH_2O)_2H \qquad (11) \\
| \\
CH_2-O-CH_2CH_2CH_2-S-CH_2CH_2-NH_2
\end{array}
$$

[0051]

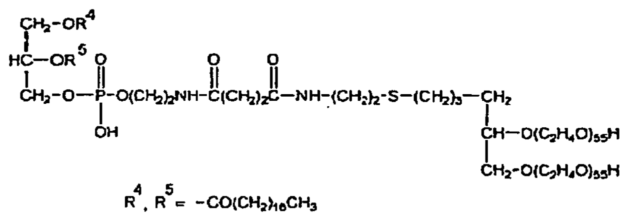
(molecular weight: 3159).

[0052]

Test Example 1

Using a fat emulsion prepared by mixing 200 g of purified soybean oil as an oil component, 4 g of dexamethasone palmitate (hereinafter referred to as DPAL) as a drug, 5 g of distearoylphosphatidylethanolamine represented by the formula (12) (hereinafter referred to as PEG2-DSPE), which was subjected to PEG modification with the compound of the formula (9) obtained in Example 1, and 7 g of yolk lecithin (hereinafter referred to as YPL), the fat emulsion was administrated into rat vein (200 mg TG/kg body weight) and a DPAL level in blood plasma was measured by liquid chromatography. Results are shown in Fig. 6.

[0053]



(12)

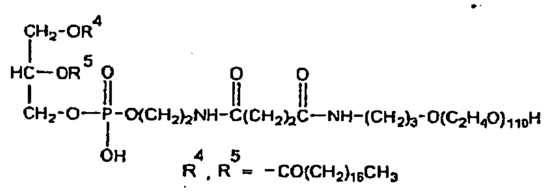
[0054]

Comparative Test Example 1

Operations were conducted in the same manner as in Test Example 1 except that 5 g of distearoylphosphatidylethanolamine (hereinafter referred to as PEG1-DSPE) represented by the formula (13) modified with single-chain polyethylene glycol was used instead of the compound (12) used in Test Example 1. Results are

shown in Fig. 6.

[0055]



(13)

[0056]

Comparative Test Example 2

Operations were conducted in the same manner as in Test Example 1 except that 12 g of yolk lecithin (YPL) was used without using the drug-containing fat emulsion prepared in Test Example 1 and PEG2-DSPE. Results are shown in Fig. 6. From Fig. 6, there was obtained a result that the DPAL level in blood plasma was kept high in the order of PEG2-DSPE>PEG1-DSPE>YPL.

[0057]

[Advantage of the Invention]

The compound of the present invention is a compound having polyoxyalkylene chains at α- and β-positions of glycerin and having an amino group at γ-position thereof. Owing to the presence of the amino group at the terminal, the compound can easily react with a phospholipid which is a component of a fat emulsion and a liposome forming a part of a drug delivery system. Moreover, the compound of the invention achieves, through modification of a

phospholipid, not only improvement of immunogenicity of an anticancer agent such as adriamycin or cisplatin included therein and stabilization thereof but also an effect of extending the residence time in a blood and has a low toxicity, as well as by-products are formed in small amounts.

[Brief Description of the Drawings]

[Fig. 1]

The drawing shows an infrared absorption spectrum of the compound obtained in Production Example 1.

[Fig. 2]

The drawing shows a differential and integral molecular weight distribution curve on GPC of the compound obtained in Production Example 1.

[Fig. 3]

The drawing shows a differential and integral molecular weight distribution curve on GPC of the compound obtained in Production Example 2.

[Fig. 4]

The drawing shows a differential and integral molecular weight distribution curve on GPC of the compound obtained in Production Example 3.

[Fig. 5]

The drawing shows an infrared absorption spectrum of

the compound obtained in Example 1.

[Fig. 6]

The drawing shows evaluation results of the DPAL levels in blood plasma obtained in Test Example 1 and Comparative Test Examples 1 and 2.

Fig. 1

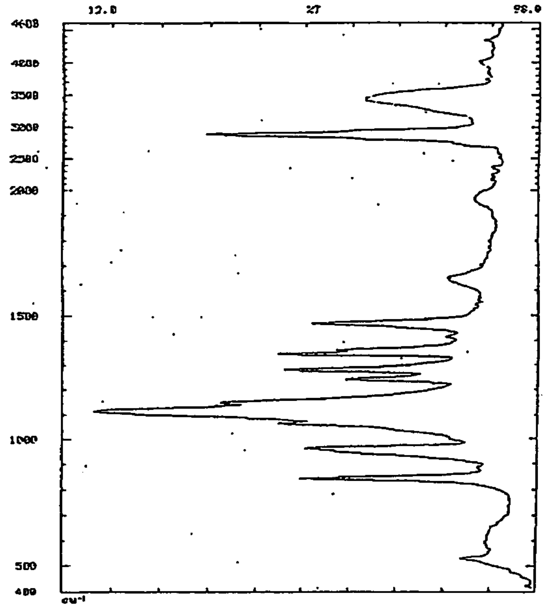


Fig. 2

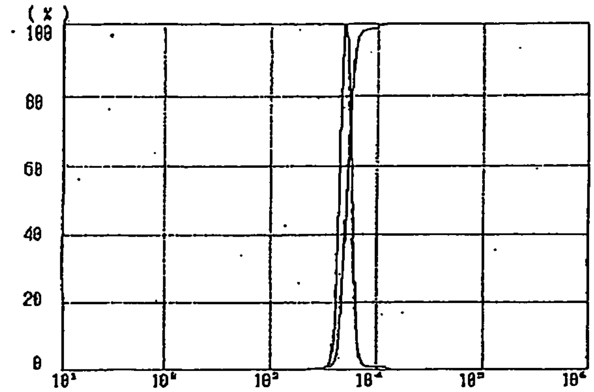


Fig. 4

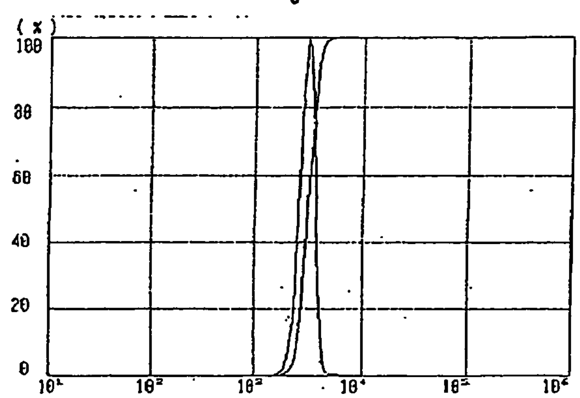


Fig. 3

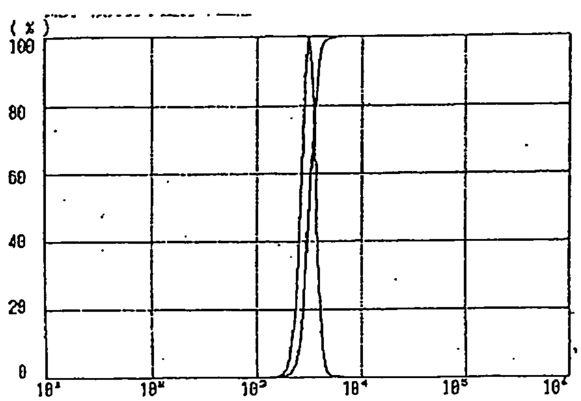


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Fig. 5

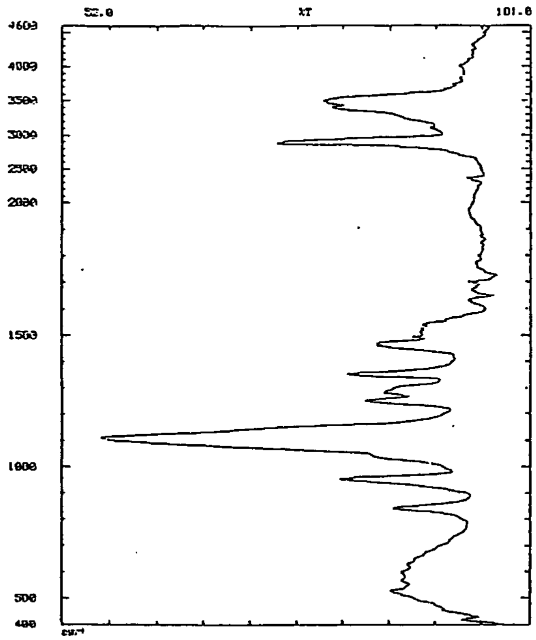
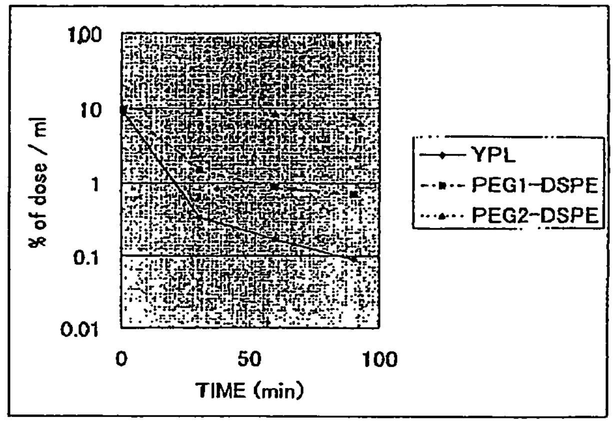


Fig. 6



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